

XX. *On a certain Excretion of Carbonic Acid by Living Plants.* By J. BROUGHTON, B.Sc., F.C.S., Chemist to the Cinchona Plantations of the Madras Government. Communicated by J. D. HOOKER, M.D., F.R.S.

Received March 31,—Read April 29, 1869.

WHILE I was engaged in some experimental determinations of the changes that take place in the composition of the Cinchona barks after being taken from the tree, a somewhat singular circumstance was noticed. Some freshly gathered barks being placed in a dish over water and covered with a bell-jar, in order to prevent loss of weight by evaporation, it was noticed that after a few hours the level of the water became depressed in the jar, and that frequent bubbles of gas escaped through the water. When it is remembered that the peculiar tannin of the cinchonæ absorbs oxygen from the air, the increase in the volume of the air appeared unaccountable.

A direct experiment was therefore made, in which 5 grms. of the fresh bark of *C. officinalis* were placed with 11·2 cub. centims. of oxygen over mercury and allowed to remain for twelve hours' darkness, and seven hours' daylight. At first a considerable absorption took place, but soon gas was given off. When the mercury stood at its original level, the gas was analyzed. It consisted of 9·7 cub. centims. of carbonic acid, 1 cub. centim. of oxygen, and 0·5 cub. centim. of nitrogen.

A similar trial was made in which 5 grms. of bark was kept in darkness in 11·5 cub. centims. of oxygen for the same period. The volume of the gas was but 9·6 cub. centims., of which 9·1 cub. centims. was carbonic acid, and 0·5 cub. centim. was nitrogen.

These two trials merely showed that the bark, like other vegetable tissues, absorbed oxygen and exhaled carbonic acid, and that in the second case the absorption had occurred in larger amount than the evolution of carbonic acid.

As these experiments did not account for the increase in the volume of the gas in my first observation, the trial was made of placing, as rapidly as possible, freshly cut strips of bark over mercury in a eudiometer. It was soon observed that gas was constantly given off from the bark, by the mercury descending in the eudiometer. This gas, on being analyzed, was found to be nearly pure carbonic acid. The evolution of gas continued for many days. When the bark was removed it was found perfectly fresh and healthy, and exhibited none of the reddening which is so characteristic of the Cinchona barks after having been exposed to the influence of oxygen; but as soon as it was brought into contact with the air, the greenish tint of the cut surface rapidly became brownish-red. The 5 grms. of bark had given off more than double its volume of carbonic acid, under circumstances which entirely precluded the possibility of its being produced by the direct oxidation of any constituent of the bark tissues.

The above conclusions led to an extension of the observations. As in the above case the gas was evolved under diminished pressure, experiments were tried in which the eudiometers containing the bark floated in vessels containing mercury, so that the gas was evolved under a slight increase of pressure. The gas, however, was given off in about the same proportions, and the floating eudiometers gradually rose. Other trials showed that though the slicing of the bark at first facilitated the evolution of gas, after seven days the amount was the same, whether the bark was put in whole or cut up into pieces.

In order to determine the amount and composition of the gas given off by the bark at different times, it was evidently necessary to operate upon a larger quantity than 5 grms. The plan was adopted of placing 47 grms. in a bottle connected with a Sprengel's air-pump (fig. 1); the precaution recommended by FRANKLAND* being taken—namely, that of immersing all joints in liquid, to preclude any possibility of leakage. The bottle of bark was at first carefully exhausted, and allowed to remain so for six hours, and after that lapse of time again carefully exhausted, so that the gas evolved from the bark might sweep every trace of adventitious gas that might be present. These operations were completed at dusk of evening. Subsequently the gases evolved were pumped off every twelve hours, and collected in a eudiometer over mercury, measured, and analyzed. The following Table gives the results, in cubic centimetres, corrected for temperature, pressure, and moisture.

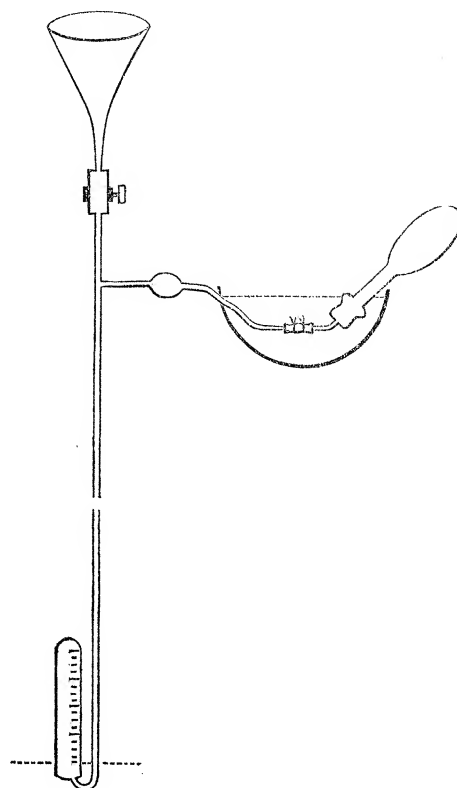


Fig. 1.

	Gas evolved at night.			Gas evolved by day.		
	Total gas.	CO ₂ .	N.	Total gas.	CO ₂ .	N.
1st day	18.22	17.51	0.71	26.07	25.66	0.41
2nd „	21.93	21.41	0.52	19.40	18.90	0.50
3rd „	15.84	15.51	0.33	16.23	16.02	0.21
4th „	13.74	13.30	0.44	13.23	13.06	0.17
5th „	11.57	11.40	0.17	12.17	12.09	0.08
6th „	9.28	9.28	0.00	12.94	12.94	0.00
7th „	7.39	7.39	0.00	10.72	10.72	0.00
8th „	8.50	8.50	0.00	9.14	9.14	0.00
9th „	7.44	7.44	0.00	8.30	8.30	0.00
10th „	4.51	4.51	0.00	5.21	5.21	0.00
11th „	3.20	3.20	0.00	3.12	3.12	0.00
12th „	3.47	3.47	0.00	3.93	3.93	0.00
13th „	1.69	1.69	0.00	2.04	2.04	0.00
14th „	1.50	1.50	0.00	2.13	2.13	0.00

* Journal of the Chemical Society, 2nd series, vol. vi. p. 90.

It therefore appears that during fourteen days the bark had evolved 269 cub. centims. of carbonic acid, or more than four times its own volume, under circumstances which preclude the absorption of oxygen. The Table also indicates a preponderance of the evolution during the day (a circumstance which other observations show in a marked manner). The amount exhaled during the day amounted to 143 cub. centims., that exhaled during night 126 cub. centims. The small amounts of nitrogen are probably due to some of that gas being obstinately retained in the tissues of the bark.

Other parts of various plants were submitted to the treatment described above, of exposure in eudiometers over mercury. In all cases gas was evolved. This gas did not consist, in most cases, wholly of carbonic acid, but contained nitrogen, though in far less amount. The residual gas, after the removal of the carbonic acid, was in several cases tested for oxide of carbon, and for marsh-gas; but in no instance could a trace of either be detected. The following Table contains an account of many of the experiments of this nature. The gases are reduced to the usual standards, and corrected for aqueous vapour.

Name and part of plant.	Weight, in grms.	Weight, in grms., dried.	Total gas, cub. centims.	CO ₂ , cub. centims.	N, cub. centims.	CO ₂ from 1 grm. nat. state.	CO ₂ from 1 grm. dry.	Time of experiment.
Bark of <i>Cupressus Lusitanica</i>	5·00	17·90	15·00	2·00	3·00	5 days.
Leaf of <i>Cinchona officinalis</i> , cut in pieces	0·84	0·28	0·68	0·68	0·80	2·40	5 "
Leaf of <i>Cinchona officinalis</i> , with petiole sealed	1·30	0·43	0·90	0·69	2·07	5 "
Leaf of <i>Mahonia Leschenaultii</i>	0·65	1·70	1·31	0·39	2·01	5 "
Wood of <i>Cinchona officinalis</i>	5·00	2·75	1·20	0·70	0·50	0·14	0·25	5 "
Twig with leaves of <i>Cinchona officinalis</i> , stalk sealed	4·75	1·50	6·90	6·62	0·28	1·39	4·41	4 "
Leaves of dwarf bamboo	10·00	3·25	5·55	4·83	0·72	0·48	1·48	5 "
Blades of <i>Festuca ovina</i>	10·00	2·68	21·97	19·51	2·46	1·95	7·28	5 "
Rhizome of <i>Iris Germanica</i>	4·75	1·13	11·70	11·10	0·60	2·33	9·82	5 "
Spadix of <i>Richardia</i>	3·00	0·50	9·35	8·75	0·60	2·91	17·50	5 "
Pseudo-bulb of an orchis	5·25	1·25	1·60	1·50	0·10	0·28	1·20	5 "
Rootlets of <i>Acacia dealbata</i>	10·00	2·00	5·70	5·00	0·70	0·50	2·50	5 "
Rootlets of <i>Festuca ovina</i>	10·00	2·75	25·80	24·60	1·20	2·46	8·94	5 "
Woody stems of <i>Erica pubescens</i>	5·00	2·50	11·90	11·30	0·60	2·26	4·50	5 "
Leaves of <i>Erica pubescens</i>	5·00	1·25	7·90	5·80	2·10	1·16	4·64	5 "
Leaves of <i>Cupressus Lusitanica</i>	10·00	4·75	13·50	12·60	0·90	1·26	2·65	5 "
Phyllodes of <i>Eucalyptus globulus</i>	5·00	2·50	10·50	8·90	1·60	1·78	3·56	5 "

Among a larger number of experiments, conducted with various portions of phanerogamous plants, I have not found a case in which carbonic acid failed to be given off, in greater or less amount, when they were exposed in a tube over mercury. Even mosses and liverworts show the same reaction, though in many instances the quantity exhaled is but small. A plant of *Jungermannia*, however, gave off absolutely no gas for three days; but at the end of that time a small quantity appeared. This was found to be carbonic acid. The probable reason why these lower orders of plants, rich as they are in chlorophyll, and in active growth, evolve in many cases a less amount of gas, is indicated in the general conclusions of this memoir.

The actual amount of gas evolved from different plants or different parts of the same

plant varies very considerably. Generally speaking, it seems to be greater according to the activity of the plant's growth. It is also given off at a greater rate when the temperature is increased. Daylight also has a stimulating effect, which is sometimes very marked, and is irrespective of temperature*.

The phenomenon is entirely dependent on the life of the tissues. Causes which arrest life also arrest the exhalation of carbonic acid. Immersion in water at 60° C. for two minutes entirely deprives the part of the property, whether it be bark, leaves, flower, or roots. Sudden drying *in vacuo* over sulphuric acid has the same effect, even when the plant is allowed to imbibe water till it has the appearance of life. In both cases it will remain for days without gas appearing in the eudiometer. Even living parts of plants in a state of rest give off no gas. Such is the case with the tuber of a potato.

It will be observed in the foregoing tabular statement that in most cases nitrogen gas is also given off. In the greater part of these it is due to the tissues of the plant containing air. This was experimentally proved in the case of the phyllodes of *Eucalyptus globulus*. It will be seen in the Table that 5 grms. of these gave off 1·60 cub. centim. of nitrogen. But when the phyllodes were put into a vessel connected with the mercurial pump in the manner before described, and thoroughly exhausted, the gas subsequently exhaled was pure carbonic acid. In some other cases it was found impossible to introduce leaves &c. into the eudiometers without some adherent air, and hence the source of nitrogen. But from Fescue grass nitrogen was actually evolved in considerable quantities. It was remarked that the grass altered its colour in the eudiometer, and, unlike most plants, suffered by the treatment to which it was subjected. To ascertain more fully the conditions of this exhalation of nitrogen, two similar bottles were filled with blades of the grass. One was placed in connexion with the Sprengel pump, the other served for occasional examination. The following numbers were obtained from 85 grms. of grass.

Gas of day.			Gas of night.			
Total gas.	CO ₂ .	N.	Total gas.	CO ₂ .	N.	
54·5	54·5	0·00	30·9	30·9	0·00	} No decomposition apparent. Faint smell of decay. Fermentation set in.
28·2	26·79	1·41	24·2	20·32	3·88	
36·5	31·50	5·00	32·8	27·67	5·13	
34·0	26·73	7·27	46·5	38·73	7·77	
38·5	29·92	8·58	44·2	35·91	8·29	

Hence from grass it will be observed that the evolution of nitrogen continually increased in amount, and was coincident with the setting in of decomposition. I am I think justified in attributing the production of nitrogen to the breaking up of the nitrogenous constituents of the plant. The grasses are exceptionally unsuitable subjects for this kind of investigation, from the readiness with which they begin to ferment when in

* Leaves immersed in carbonic acid have been shown by BOUSSINGAULT (Compt. Rend. vol. lx. p. 872) to have no power of decomposing the gas under the influence of light.

mass. Many kinds of leaves with which I have experimented appeared quite fresh and unchanged after fifteen days' confinement in the eudiometer.

When the investigation had arrived at this stage, it became necessary to consider the somewhat exceptional conditions under which the experiments were made. The plants grew and were experimented with on the Neilgherries at a height of 7400 feet, or 1·4 mile up in the air. Hence the gases would meet with less mechanical impediments to their evolution than at the sea-level. To ascertain whether this circumstance had any essential effect on the phenomena, soda-water bottles were filled with leaves of *Erica*, fresh Cinchona bark, &c., and connected with manometer-tubes containing mercury (fig. 2). Soon the mercury began to rise, being pressed upwards by the gas exhaled, until the total pressure had become equal to 30 inches. Then it continued to rise several inches higher. In one case a final pressure exceeding a whole additional atmosphere was reached. The leaves were perfectly healthy, and showed no trace of any decomposition. In addition to these experiments others were made, in which the bottles filled with leaves &c. were fitted with delivery-tubes, whose ends were plunged a few inches below the surface of mercury. The gas was collected in a eudiometer. The results confirmed the other observations. This form of the experiment is well suited for a lecture illustration. Hence it is concluded that variations of atmospheric pressure within wide limits do not essentially affect this peculiar evolution of carbonic acid.

All the foregoing experiments were performed with portions of plants separated from the root, and therefore under somewhat abnormal conditions. The important question arises, whether the same phenomenon constantly occurs in plants growing under ordinary conditions. Although there could be *à priori* little doubt of this, an experimental proof was desirable. It is one that offers many practical difficulties, and I have endeavoured to effect it as follows.

I was first anxious to detect the exhalation of carbonic acid from growing Cinchona bark. As a preliminary qualitative experiment, I took a healthy young plant of *C. officinalis*, about two years old; and by plucking off all the leaves from the stem, except those of the terminal shoot, it was enabled to be passed into a tube of glass, resembling the outer tube of a Liebig's condenser (fig. 3), which was then made air-tight at each end by means of sheet caoutchouc and cement. The intervening space, between the enclosed bark and the wall of the tube, was filled with hydrogen and allowed to remain for a day. It was then cleared out by a stream of hydrogen, and the upper branch tube conveying the hydrogen tied. The lower one had

Fig. 2.

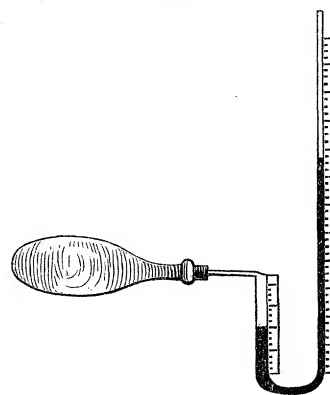
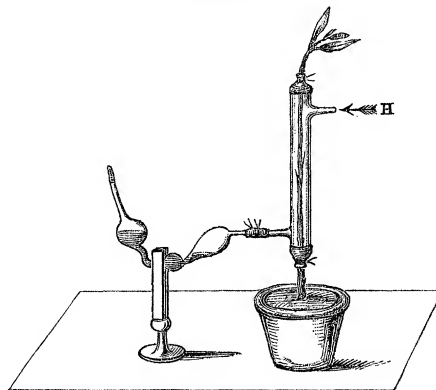


Fig. 3.



been arranged to deliver any gas that might be evolved into a eudiometer over mercury. But the gas was delivered in a very unsatisfactory manner, owing to the pressure that had to be overcome before the gas could pass into the eudiometer. At the end of two days, however, a few bubbles had passed, which, when tested by a potash pellet, showed the presence of carbonic acid by a marked absorption.

The plan was then adopted of passing a slow stream of hydrogen (well purified by passing through tubes of pumice moistened with a strong solution of argentic nitrate and through a pair of potash bulbs) through the intervening space and then through a nitrogen bulb containing baryta-water. In this way the constant evolution of carbonic acid by the bark was readily detected by the precipitation of baric carbonate. The experiment was continued for nine days. The last three days the amount was estimated: the vols. of carbonic acid, corrected to the ordinary standard of pressure, were as follows:

7th day	2.10 cub. centims.
8th „	1.54 cub. centim.
9th „	1.64 cub. centim.

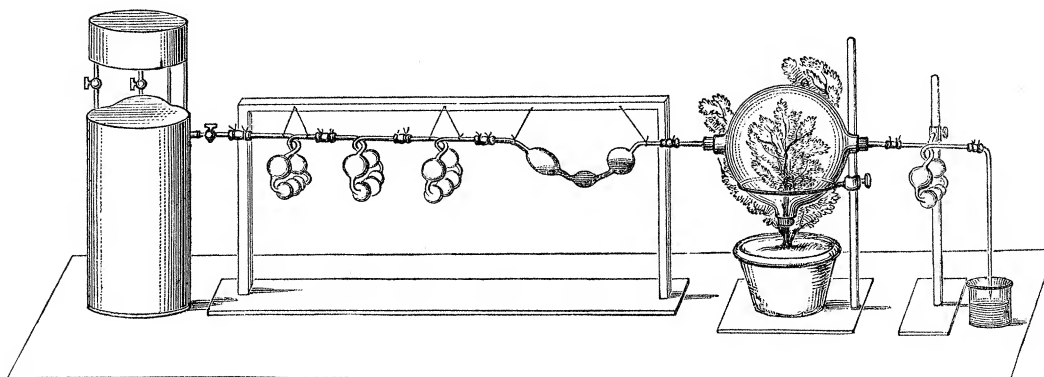
Immediately after the conclusion of the experiment, the bark, which had for so long been in contact with hydrogen only, was quickly cut off the plant, and as rapidly as possible passed into a eudiometer over mercury. Carbonic acid soon made its appearance, and after three days its amount was estimated. Its corrected volume was 6.21 cub. centims. The surface of the bark included in the tube exposed a surface of 18 square inches.

In applying the same method for the purpose of ascertaining the evolution of carbonic acid from leaves while growing on the plant, very considerable difficulty was experienced in continuing the experiment long after the exclusion of oxygen. This was owing to the plant suffering from the effects of the hydrogen employed. This gas, when generated in the usual manner from zinc and sulphuric acid, even after the most careful purification I could devise, had such a poisonous effect on the fresh leaves, that after three days' exposure they became yellow and died. I am inclined to attribute this to some impurity in the gas (as a trace of arsenic) which was not perfectly removed by the means of purification adopted. I tried several plans of purification and several kinds of plants. In all cases I found carbonic acid evolved; but I was never able to continue the experiment a sufficient length of time without the leaves becoming manifestly unhealthy, and thus introducing a source of doubt. After many trials I found that carefully washed and purified nitrogen was the gas best suited for my purpose. I found also that a branch from a plant of *Erica pubescens* appeared perfectly healthy and fresh after many days' exposure to the gas.

I therefore finally adopted the following arrangement, which will be readily understood by the annexed sketch (fig. 4). Nitrogen gas contained in the gas-holder was slowly passed through two LIEBIG'S potash bulbs containing a solution of pyrogallie acid in strong potash, then through a similar bulb containing a solution of potash, after this through baryta-water in a WILL'S nitrogen bulb (which remained clear to the end). The pure gas then passed into the three-necked globe containing the branch of *Erica*, the latter

being secured in one neck by means of a perforated stopper of soft caoutchouc with a side slit for the introduction of the stem, so that, being firmly thrust into the neck, it

Fig. 4.



made an air-tight joint. The issuing gas then slowly bubbled through a potash bulb, which was periodically weighed—or else, when a test was required, through baryta-water. Contamination with the external air was prevented by finally allowing the gas to go into distilled water.

The gas was passed before regular determinations were made. During this period occasional tests were made with baryta-water, which always showed the presence of carbonic acid. On the seventh day the gas was passed through the potash bulbs, which were weighed night and morning. The following were the results:—

	By day, weight in grammes.	By night, weight in grammes.
7th day	0·0186	0·0202
8th „	0·0362	0·0532
9th „	0·0375	0·0335
10th „	0·0395	0·0133
11th „	0·0372	0·0152
12th „	0·0183	0·0125
13th „	0·0566	0·0147
14th „	0·0089	0·0194
16th „	0·0464	0·0194
17th „	0·0186	0·0016
18th „	0·0210	0·0121

The foregoing numbers indicate that the branch of *Erica* evolved carbonic acid long after being excluded from the influence of oxygen. The mean amount during the last eleven days was somewhat over 1 cub. centim. per hour. In this experiment the exhaled carbonic acid, being mingled with a large amount of inert nitrogen, was liable to be decomposed by the plant during day. This circumstance was doubtless a source of loss of carbonic acid, but it was unavoidable.

The excretion of carbonic acid by plants has generally been ascribed to a process analogous to the breathing of animals, oxygen being absorbed and carbonic acid exhaled. The greater part of the carbonic acid exhaled has doubtless this origin, but it is clear from the foregoing experiments that it will not account for the whole. They show that

plants, when placed in circumstances where access of oxygen is no longer possible, continue to give off carbonic acid, in quantities and under conditions which involve the conclusion that it is a necessary function of the life or growth of the plant.

There appear to be but two ways of accounting for this excretion of carbonic acid:—1st. It may possibly be due to absorption of oxygen occurring long prior to the exhalation. 2nd. It may be due to the direct giving off of carbonic acid in consequence of the chemical changes occurring in the plant. It may also be a consequence of both conjointly.

If it were possible to exclude oxygen from a plant during the whole of its life, or if a plant could be grown for some months in a mixture of neutral gas and carbonic acid, the exhalation of the latter gas must necessarily be ascribed to the second hypothesis. Experiments in this direction, however, offer extreme difficulties, or are even impossible. DE SAUSSURE states that no plants will live in an atmosphere destitute of oxygen. There is, however, a doubt whether this conclusion is perfectly general.

There is, however, another plan by which considerable light may be obtained in the matter. It consists in ascertaining the quantitative effect produced on the exhalation of carbonic acid from parts of plants by their exposure to the oxygen of the air during periods of known direction. This I have endeavoured to carry out as follows.

A certain weight (usually 5 grms.) of leaves or bark was exposed to air for twenty-four hours in a bell-jar over water immediately after being separated from the plant. In the case of bark it was found necessary to do this while still attached to the wood. An equal amount, as similar in character as possible, was exposed for the same period to an atmosphere of nitrogen. Thus the two cases only differed by a day's exposure to the influence of oxygen. The effect of this was ascertained by placing each in a eudiometer over mercury, and measuring the respective amounts of carbonic acid given off each day.

The following Tables express the results obtained. The first column gives the number of days after the separation from the plant. Column marked "Air" relates to the specimen exposed to air, that marked N refers to the one placed in nitrogen. The column Δ expresses the difference of the two, or the effect of a day's exposure. The results are given in cub. centims. of dry CO_2 reduced to the ordinary standard.

Bark of <i>Litsea oblonga</i> .				Bark of <i>Cupressus Lusitanica</i> .				Bark of <i>Acacia dealbata</i> .			
I.	Air.	N.	Δ .	I.	Air.	N.	Δ .	I.	Air.	N.	Δ .
2	6.20	5.50	0.70	2	2.06	1.53	0.53	2	5.14	4.09	1.05
3	6.13	4.37	1.86	3	2.54	2.05	0.49	3	3.85	3.04	0.81
4	5.06	4.10	0.96	4	2.20	1.91	0.29	4	4.37	4.30	0.07
5	4.39	3.76	0.63	5	2.10	1.94	0.16	5	2.61	2.60	0.01
6	4.26	3.70	0.56	6	1.65	1.65	0.00	6	2.10	2.10	0.00
7	3.39	3.35	0.04	7	1.49	1.49	0.00	7	1.61	1.61	0.00
8	2.89	2.89	0.00	8	1.20	1.20	0.00				
9	2.38	2.38	0.00	9	1.05	1.05	0.00				
10	1.62	1.64	-0.02								
11	1.74	1.74	0.00								
12	1.72	1.72	0.00								
13	0.85	0.85	0.00								

The subjoined Table gives the results of a similar experiment with the leaves of *Buxus sempervirens*, which were found particularly suitable for the purpose from their activity and the hardiness with which they stand the hardships of their treatment without the least apparent injury.

Leaves of <i>Buxus sempervirens</i> .			
I.	Air.	N.	Δ.
2	7.11	5.97	1.14
3	4.74	3.98	0.76
4	4.38	2.74	1.64
5	2.79	2.40	0.39
6	1.86	1.61	0.25
7	2.32	2.07	0.25
8	1.53	1.29	0.24
9	1.22	1.13	0.09
10	1.09	1.09	0.00
11	0.95	0.95	0.00
12	0.87	0.87	0.00

Similar but less-extended results were obtained with the leaves of *Veronica salicifolia*, which appeared to suffer from their treatment. I do not, therefore, quote them.

The above Tables demonstrate the fact that exposure to air for one day markedly increases the amount of carbonic acid during many subsequent days. Hence the hypothesis (1) enunciated above expresses (partly at least) the true origin of the carbonic acid given off by plants when confined over mercury. It is really due in a great measure to prior oxidation. Some other general conclusions may be fairly deduced from this result.

It is of course well known that plants by exposure to air constantly absorb oxygen and exhale carbonic acid. Now the further fact may be added, that, of the oxygen absorbed during a day, part may not appear as carbonic acid till after the expiration of a week. This explains the circumstance noticed by DE SAUSSURE and others, that the volume of carbonic acid exhaled during a day is sometimes more and sometimes less than that of the oxygen absorbed. The actual process is not so simple as has been generally supposed. Thus, when a plant is observed during a certain day to absorb oxygen and give out carbonic acid, the greater part of the latter is due to the oxygen absorbed during that day; but some is derived from that absorbed the day previous, a smaller amount from the oxygen of the day previous to that, and so on back for several days. On the other hand, the oxygen absorbed is excreted as carbonic acid for the greater part on the same day, but by no means the whole; for decreasing amounts will be the source of part of the carbonic acid exhaled by the plant during the ensuing week.

But it is to be observed that the whole of the carbonic acid exhaled by the barks and leaves in the above experiments cannot be referred to previous oxidation. This is clearly shown by the foregoing tabular statements. Thus, in the case of the bark of *Litsæa oblonga*, it is seen that the differences gradually diminish in value and finally disappear, while a considerable amount of gas is still daily exhaled. This gas cannot be attributed

to any previous oxidation, since the very latest exposure to oxygen has ceased to be perceptible. The total daily amount of excreted carbonic acid is hence not comminuent with that which is the effect of the single day's exposure, as would be the case if the carbonic acid had no source but that of previous oxidation. It therefore is evident that some of the carbonic acid has another source.

Again, if the peculiar exhalation of carbonic acid, which I have in the foregoing pages endeavoured to investigate, were exclusively due to previous oxidation, it is obvious that the amount in column "Air" for any day should not exceed the sum of the numbers in column " Δ " for that and all subsequent days; but it does exceed this sum in all cases. This consideration corroborates the conclusion that there is another source of carbonic acid.

There is an objection to this reasoning which can be made with some justice. "How can we be certain," it may be urged, "that the result of a single day's exposure to an oxidizing atmosphere is proportionally the same as that of several consecutive days, or as in nature, where the exposure is of course continual? Perhaps in this case a *greater* proportional effect may take place, and thus the whole amount of carbonic acid may be accounted for."

This doubt, however, admits of an experimental resolution. If it be true that longer exposure causes proportionally larger amounts of carbonic acid to be subsequently evolved, then, in a differential experiment like the foregoing, if the time of exposure to air be doubled, will the sum of the differences approach *nearer* to the actual amount exhaled on any particular day?

To determine this point, an experiment resembling the preceding was made, with the difference that the box-leaves were respectively exposed to air and nitrogen for forty-eight hours instead of twenty-four. The results of the subsequent determinations were as follows:—

Leaves of Box.			
I.	Air.	N.	Δ .
3	6.40	4.95	1.45
4	6.96	5.05	1.91
5	4.88	4.55	0.33
6	3.26	2.88	0.38
7	2.65	2.33	0.32
8	1.52	1.15	0.37
9	1.45	1.15	0.30
10	0.86	0.84	0.02
11	0.76	0.76	0.00
12	0.45	0.45	0.00
13	0.45	0.45	0.00

An examination of the foregoing Table shows that the sum of the n th, $n+1$ th, $n+2$ th, &c. differences approaches *less near* the n th term in column "Air" than in the former experiment, where the exposure was but for twenty-four hours. This, however, is only true in the mean. Calculating from the first seven terms of both Tables, the sum

of differences differs from the n th term in the "Air" column by a mean of 1.49 and 1.89 respectively.

It must therefore be concluded that part of the carbonic acid exhaled from vegetable tissues arises from a source independent of oxidation. It must therefore be due to a separation of the gas from one or several of the proximate constituents of their tissues in consequence of the chemical changes therein taking place.

This is a view that is by no means novel. ROCHLEDER* and others have already advanced it as an hypothesis. Indeed the former appears inclined to attribute the evolution of carbonic acid to this cause, even in some cases where it is ordinarily regarded as a direct product of the so-called plant respiration. Moreover there are several facts in the known chemistry of plant-growth which harmonize with this view. It is easily seen that the subtraction of the elements of carbonic acid from any vegetable principle will have the result of leaving a residue richer in both carbon and hydrogen, and hence of higher force-value.

There are grounds for believing that woody tissue is derived from the starch secreted under the influence of sunlight in the leaves of the plant. But as these tissues contain a larger percentage of carbon, and have a higher force-value than starch, this change would be effected by the separation of a substance having a less force-value, as is the case with carbonic acid, in which the energy of combination is nearly exhausted. (A decomposition of this nature occurs in the slow changes by which wood is converted into lignite and coal.) The conversion of starch into fats in plants is a change which there are experimental grounds for believing to be one of constant occurrence. It has been observed that during the ripening of oily seeds the starch disappears as the oil is formed. This change can scarcely be explained without assuming the subtraction of carbonic acid as in the decomposition by which alcohol, glycerine, and fatty acids are derived from sugar in vinous fermentation. The direct conversion of sugar into wax, with the evolution of carbonic acid, is known to occur by the agency of bees.

SACHS has shown that some seeds which contain no tannin develop that substance during the first growth after germination, with a simultaneous disappearance of starch. The period at which it occurs would preclude the direct formation of tannin from external sources. The tannins contain a less percentage of oxygen than the class of carbohydrates; and their derivation from the latter, attended by the separation of carbonic acid, is an hypothesis supported by the facts of the case. Without assuming unusual or improbable decompositions, it is scarcely possible to explain such conversions, unless this separation be assumed.

The state in which the oxygen absorbed by the plant remains, until it is finally excreted in combination with carbon, is a point of great interest. In order to ascertain whether it might not possibly be *occluded* by the tissues, an experiment was performed. A considerable quantity of willow bark was placed in a flask connected with a Sprengel pump, and so arranged that it could be exhausted while heated in an oil-bath. At temperatures

* Chem. u. Phys. d. Pflanzen, 1858, p. 113 und 151.

varying from 17° to 60° C. considerable quantities of carbonic acid gas were evolved, showing that the rise of temperature markedly increased the excretion of this gas. But as soon as the temperature rose above the coagulating-point of albumen, gas quite ceased to be evolved, although the pump was kept at work and the temperature raised to 160° C. The experiment hence gave no support to the idea that the oxygen was occluded. The constituent in plants which acts in relation to oxygen as the blood-corpuscles in animals is quite unknown.

It is a rather remarkable fact that growing cinchona bark, which contains a large amount of a tannin greedy of oxygen, continually absorbs oxygen, retains it for days, and excretes carbonic acid, without in the least affecting the easily alterable tannin.

The main conclusions which are arrived at by the foregoing investigation are:—

1st. That nearly all parts of growing plants evolve carbonic acid in considerable quantities, quite independently of direct oxidation.

2nd. That this evolution is connected with the life of the plant.

3rd. That it is due to two causes—namely, to previous oxidation, resulting after a lapse of time in the production of carbonic acid, and to the separation of carbonic acid from the proximate principles of the plant while undergoing the chemical changes incident to plant-growth.